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Research article

Using DNA metabarcoding to separate natural and human provided food in wild boar diet at the northern distribution range of Europe

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Wild boar *Sus scrofa* is increasing in numbers and extending its distribution across Europe and is difficult to control due to high reproductive potential. Dietary quality is a main determinant of wild boar population dynamics, and the extent to which they rely on human-provided food provide a key to limit their distribution. Yet, we lack data on wild boar diet from northern Europe. Here we use DNA-metabarcoding of faecal samples (n = 50) to determine wild boar diet during fall and winter in Norway. We mainly aimed to quantify the extent to which wild boar relies on natural or human-provided food sources. A secondary aim was to determine whether diet varies with individual characteristics (sex, age or weight), season (winter or fall), and between the two regions with wild boar in Norway. We found a high degree of diet variability between individuals. Individuals consuming high amounts of edible fungi consumed low amounts of plant material. The (heavier) male wild boars consumed 50% more human provided food than the (lighter) female wild boars. There was no clear effect of age, season (winter versus fall), or region on diet with the sample size available. The negative correlation between plants and fungi in each sample suggests that using multiple primers targeting different taxa can provide quantitative diet information, and points to an important role of fungi (truffles) during winter and fall. The large individual variation in diet may reflect opportunistic feeding tactics in Scandinavian boreal forests, driven by a lack of acorns and few crops. Our study has relevance for understanding limitations of wild boars at their northern distribution range in Europe, and thus also provides important information for management.

Keywords: baiting, diet, metabarcoding, mushrooms, supplemental feeding, wild boar

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Introduction

Among ungulates, wild boar and feral pigs *Sus scrofa* have the highest reproductive potential (Servanty et al. 2007, Fulgione et al. 2016), and their distribution ranges are expanding in many regions of the world (Massei et al. 2019, Vercauteren et al. 2020). Wild boar feeding and rooting impact both above- and below-ground biodiversity in forests, and boars are notorious for their adverse impact on agricultural production (Wilson 2004, Herrero et al. 2006, Rutten et al. 2019). Furthermore, the role of wild boars in the transmission and persistence of African swine fever (ASF) is of growing concern in both Europe and Asia (Dixon et al. 2020). Controlling wild boar populations has therefore become an even more urgent priority (Vicente et al. 2019, Fulgione and Buglione 2022), and understanding factors limiting populations is crucial when aiming to limit the expanding wild boar distribution (Palencia et al. 2023).

Wild boars are omnivores eating a wide variation of food items, and dietary quality strongly influences their population dynamics. Their diet is usually dominated (> 90%) by plants (Ballari and Barrios-García 2014), and they typically eat at least one energy-rich food source (Schley and Roper 2003). Pulsed resources, in the form of masting years with acorns, markedly impact wild boar population dynamics in forested ecosystems in continental Europe (Servanty et al. 2009, Gamelon et al. 2017). Of particular interest for management is the relative role of natural versus intentional or unintentional human-provided food sources. Feeding increases the reproductive output of wild boar, and banning feeding therefore provides one avenue towards limiting their populations.

In Scandinavia, wild boars were locally extirpated more than a 1000 years ago in Norway (Rosvold et al. 2010), while persisting up to the 1800s in Sweden before extinction (Jonsson 1986). The current population in Sweden was founded by escapees (Lemel et al. 2003). Around 1990, it was assumed to be ~ 500 wild boars, but the population has since then grown rapidly and the annual harvest now exceeds 100 000 in Sweden (Supporting information). These wild boars have recently expanded their range into Norway. Transboundary populations can become particularly challenging when management aims differ across the border, as with large carnivores (Bull et al. 2009, Bischof et al. 2020). Wild boar is considered native and a popular game species in Sweden, and there is no goal to contain the wild boar population. In contrast, Norway consider wild boar a non-native species, and the Norwegian Biodiversity Information Centre identify them to constitute an ecological risk factor (VKM et al. 2018).

The national government in Norway have developed an action plan aiming to keep 'as few wild boar as possible in the minimum possible range' (Norwegian Environment Agency and Norwegian Food Safety Authority 2019). As part of this action plan, Norway has implemented a ban on feeding wild boar, while using baiting for hunting is allowed. In contrast, the management regime in Sweden involves extensive intentional feeding. The distribution of wild boar in Norway is assumed to be more limited by food than climate, and it has been argued that farming and supplementary feeding have allowed the wild boar to persist in otherwise inhabitable areas (Rosvold and Andersen 2008). However, there have been no prior studies of wild boar diet in these northern latitudes, which is crucial for understanding population limitation.

Conventional diet analysis has relied primarily on direct observation of feeding behaviors and microscopic analysis of faecal matter to identify diet items based on morphological characters (Putman 1984). However, methodological advances in high-throughput sequencing have heralded a revolution in dietary analysis, with metabarcoding of faecal samples allowing non-invasive, species-level characterization of highly- or even fully-digested diet items on unprecedented scales (Robeson et al. 2018, de Sousa et al. 2019, Tercel et al. 2021). Nevertheless, faecal metabarcoding does not provide a perfectly accurate analysis of diet, and biases and limitations inherent to the method must be taken into consideration when interpreting its results. First, faecal-based diet analysis in general, i.e. both morphological- and molecular-based, provides a snapshot of a particular individual's diet over a limited period just prior to the faeces being deposited. The ensuing data can be stochastic and noisy without repeated sampling from the same individual. Second, faecal-based diet analysis is inherently biased due to differences in digestibility (Putman 1984), meaning the observed abundance of a diet item will be a product of both the amount consumed and the rate at which its DNA or cellular structure is broken down in the animal's gut. Third, abundant or frequently occurring diet items are not automatically important for energy budgets. Some consumed species may be 'accidental bycatch' when foraging, and some detected taxa may be contaminants introduced during sample collection. Nevertheless, faecal-based diet analysis using molecular methods provides the opportunity for high-throughput characterization of an organism's diet and is increasingly relied on for diet analyses, particularly in instances where observation opportunities are limited. As wild boars exhibit nocturnal behaviors and avoid human contact, they are particularly well suited to diet analysis using faecal metabarcoding.

We use metabarcoding of faecal samples of wild boar harvested by hunters during fall and winter in Norway to provide an initial assessment of important food items during what is assumed to be the most resource-limited time of year for the animals. Our main objective was to quantify the extent to which wild boar in Norway rely on natural or human-provided food sources. A secondary objective was to determine whether this varies with individual characteristics (sex, age or weight), season (fall versus winter) and between the two main regions with wild boar in Norway.

Material and methods

Study areas

The study area comprises municipalities Aremark (59°14′46.349″N, 11°40′57.958″E) and Halden (59°7′58.786″N, 11°23′14.845″E) in Viken county (termed

the 'south region') and Elverum (60°52′55.463″N, 11°33′44.885″E), Våler (60°39′56.048″N, 11°50′ 55.518″E), and Kongsvinger (60°11′32.795″N, 11°59′55.208″E) in Innlandet county (termed the 'north region', Fig. 1). These regions currently form the two core areas of wild boar in Norway (Odden et al. 2023). In addition, opportunistic sampling of dispersers yielded four samples outside of the main distribution range (termed 'dispersers'); from Tydal, Klæbu (from 2020 part of Trondheim) and Verdal in Trøndelag county and Nesbyen in Innlandet county.

The south region has a mild and oceanic influenced climate with shallow snow cover during winter. The terrain is mildly undulating with many lakes, and there is a mixture of agricultural areas and boreo-nemoral forests. The north region has a typical dry and cold inland climate with more long-lasting snow cover. Habitat is boreal forests intermixed with agricultural areas, and the most common crops in both regions are grains (barley *Hordeum vulgare*, wheat *Triticum aestivum*, oats *Avena sativa* and some rye *Secale cereale*). While there is also field production of vegetables (potatoes are common), there is limited cultivation of maize. There are roe deer *Capreolus capreolus*, moose *Alces alces* and scattered populations of red deer *Cervus elaphus* in both regions. The mycoflora of the regions is not thoroughly characterized, but epigeous macrofungi are abundant, and recent studies have demonstrated that a diverse array of hypogeous truffles occur

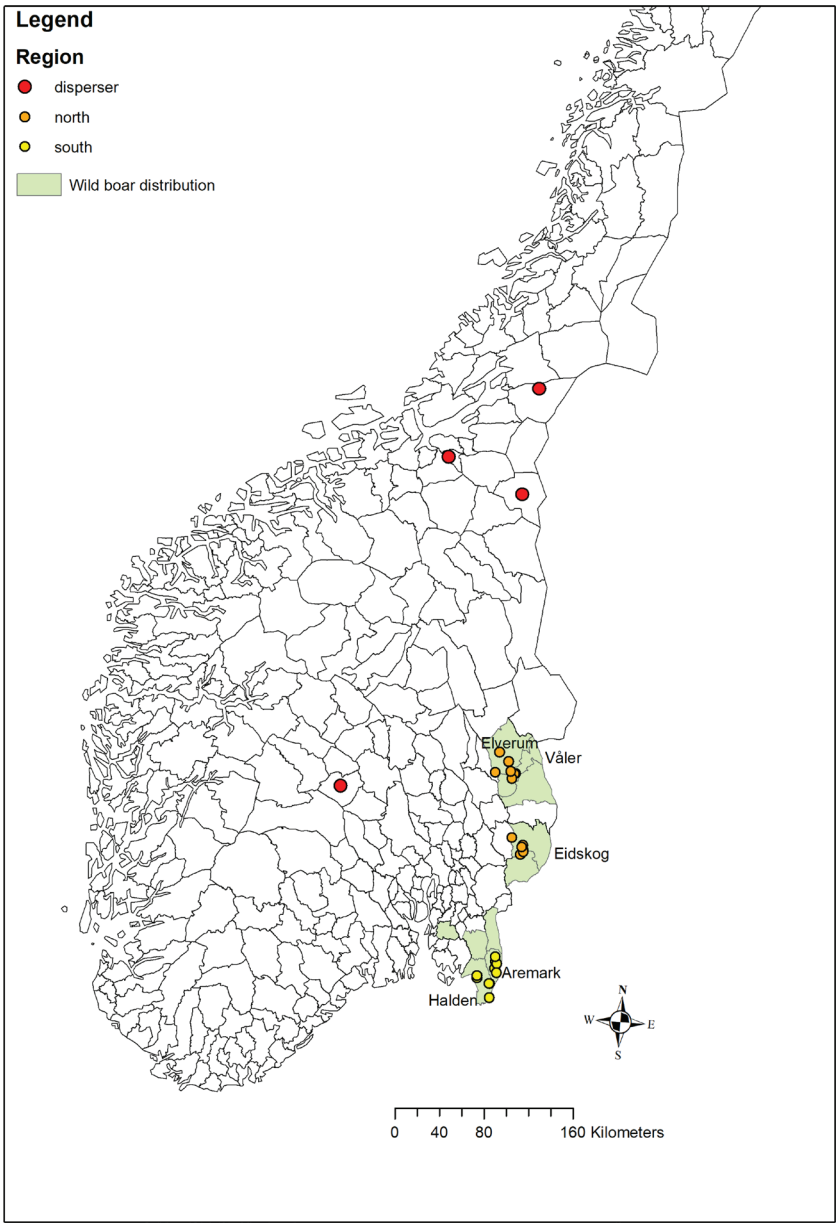

Figure 1. A map of the distribution range of wild boar in south Norway. The samples derive from the southern and northern region and from four dispersers outside of main distribution range.

at high frequencies throughout Norway (Molia et al. 2020, Norwegian Biodiversity Information Center 2023).

Data collection and stratification

Since there is a risk of contracting trichinosis and salmonella from eating wild boar meat, the Norwegian Food Safety Authority subsidizes laboratory analysis so that hunters get free testing for those diseases if they provide samples (sent to Norwegian Veterinary Institute for microbiological analysis and to Norwegian Institute for Nature Research for metabarcoding). The faecal samples analysed here (n = 50) were collected by recreational hunters from October 2020–March 2021, except for four samples from dispersers that included October and November 2019 (Table 1). Hunters were provided with sampling kits containing a 40 ml sample container filled with approximately two-thirds silica, and a wooden teaspoon to take the sample from the rectum/intestine. The amount they were asked to take was equivalent to a teaspoon, so that the silica could absorb the moisture to the greatest extent possible. The hunters then sent the sample by mail, after which the sample container with faeces and silica was stored in a −20°C freezer, and in addition a minimum of 3–4 days in a −80°C freezer as part of standard precautionary measures in the lab to prevent zoonoses. Hunters reported data on date of harvest, sex, age, body weight (before and after butchering), and either exact UTM-position or name of location, and they are used to report similar data (Cretois et al. 2020). To get positions for our map (Fig. 1), we searched on name of location in Google Maps™ mapping service and extracted coordinates. From incoming samples, we stratified the 50 chosen samples sent to processing on region (23 from each), age (26 juveniles, 23 adults, 1 uncertain) and sex (30 males, 20 females), and included all four dispersing individuals.

DNA isolation and sequencing

Entire faecal samples were placed in 50 ml centrifuge tubes containing a mixture of 1.4 mm ceramic spheres, 0.1 mm silica spheres, and 4 mm glass beads (Lysing Matrix E, MP Biomedicals) and homogenized by shaking at 6 m s^{-1} for 40 s. The MP FastDNA™ SPIN kit for soil (50 ml) was used to isolate DNA from the homogenized material, with the omission of the initial three steps intended to remove humus and litter from soil samples. DNA concentration and quality were assessed using Nanodrop and only samples with a 260/280 absorbance ratio > 1.5 were analyzed further. Amplicons were generated for gene regions targeting three different potential diet components for wild boar: 1) the ITS2 region of rDNA for plants was targeted using the plant-specific ITS-S2F (Chen et al. 2010) and ITS4 (White et al. 1990) primers, 2) the ITS2 region of rDNA for fungi was targeted using the fungal-specific fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990) primers and 3) the COI region of mitochondrial DNA for metazoans using the MICOIintF (Leray et al. 2013) and PolyShortCoiR (Carr et al. 2011). For all markers, single PCR reactions were conducted in 25 µl volumes with 25 ng of template DNA and contained 1× KAPA HiFi HotStart ReadyMix (Roche, Switzerland). Primer concentrations were 0.5 µM for fungal amplicons, and 1 µM for plant and metazoan amplicons. PCR reactions for the metazoan COI marker also included 1 µM of a pig COI blocking primer (Robeson et al. 2018) to reduce signal from wild boar DNA. A negative isolation control to which no faecal matter was added and negative PCR control using DNA-free water instead of template were included for each marker, and for the metazoan marker, a positive PCR control of wolf tissue *Canis lupus* was included. PCR-conditions consisted of an initial denaturing step of 5 min at 95°C, followed by 35 cycles of 30 s denaturing (95°C), 30 s annealing (ITS: 56°C, COI: 55°C), and 30 s extension (72°C) with a final elongation step of 5 min at 72°C. PCR products were quantified using Tape Station (Agilent 4200) and cleaned of excess primers and nucleotides using magnetic beads (MAG-BIND RXN PURE PLUS) to select fragments between 200–600 bp in length (ITS amplicons) or over 250 bp in length (COI amplicons). The size-selected amplicons were used as a template for a second, indexing PCR using the Nextera XT Index kit (Illumina, USA) according to the manufacturer's instructions. The indexed samples were again cleaned as described above, pooled in equimolar amounts, and sequenced in a paired end 250 bp run with SP ver. 1.4 chemistry on the Illumina NovaSeq 6000 sequencing platform at the Norwegian Sequencing Centre (NSC), University of Oslo (UiO), Oslo, Norway.

Bioinformatic analyses

Samples were demultiplexed, and indices and adapters removed on the Illumina NovaSeq sequencing platform by the NSC. Primer sequences were identified and removed

Table 1. An overview of sample size per month (2020–2021) and municipality in county Viken (above dotted line) and Innlandet of wild boar faeces from Norway. For location of dispersers, compare Fig. 1.

Region	Municipality	October	November	December	January	February	March	Sum
South	Aremark	2	4	1	2	3	1	13
	Halden	2	1	3	2	2	0	10
North	Våler	2	2	0	1	0	1	6
	Kongsvinger	1	1	0	3	8	0	13
	Elverum	0	2	1	0	0	1	4
NA	'Dispersers'	1	1		2			4
Sum		8	11	5	10	13	3	50

from both the 5′ and 3′ ends of forward and reverse reads using cutadapt ver. 1.9.1 (Martin 2011), allowing up to 15% mismatch across the length of the primer. The DADA2 ver. 1.19 package for R (Callahan et al. 2016) was used for further quality filtering, error correction, and chimera detection. Reads were quality filtered to remove all sequences with ambiguous bases, > 2 expected errors in the forward direction and reverse directions, and length < 50 bp after truncation at the first instance of a base with quality score < 10. For the COI amplicons, all reads were truncated at a length of 210 bp. Error rates were estimated for forward and reverse sequences with enforced monotonicity, forward and reverse reads were merged with a minimum overlap of 30 bp, and amplicon sequence variants (ASVs) were inferred for each sample. Chimeric sequence variants were assessed on a per-sample basis, as chimeric events occur at the individual PCR-level. If a sequence variant was flagged as chimeric in more than 90% of the samples it occurred in, it was removed.

For the ITS2 marker for plants, taxonomy was assigned using the SINTAX classifier (Edgar 2016), as implemented in 'vsearch' ver. 2.14.1 (Rognes et al. 2016) against 1) the global PLANiTS database (Banchi et al. 2020) and 2) a custom database comprising publicly available reference ITS sequences for all plants listed as occurring in Norway in the Norwegian Biodiversity Information Center's Species Nomenclature Database (Artsdatabanken 2015). Minimum bootstrap confidence values of 80% were required for a successful taxonomic assignment at a given level, and the assignment at the lowest taxonomic level that was parsimonious between the two databases was accepted. Each ASV was also subjected to a BLAST search against the NCBI nucleotide non-redundant database. Any ASV with a best BLAST match to a lineage outside Streptophyta, or that could not be assigned with confidence > 80% at the order level, was designated a non-target amplification and excluded from further analyses. Plant species were then classified as agricultural, horticultural, or native based on their occurrence in Norway (Supporting information).

For the ITS2 marker for fungi, taxonomy was assigned using the IDTAXA algorithm with a training set based on the UNITE database (Nilsson et al. 2018). ASVs were also subject to a BLAST search against the NCBI nucleotide non-redundant database. Any ASV with a best BLAST match to a lineage outside kingdom Fungi, or that could not be assigned with confidence > 80% at the order level was designated a non-target amplification and excluded from further analyses. Fungi were then classified using their assignments in the FUNguild database (Nguyen et al. 2016) as truffles, macrofungi, microfungi, yeasts and coprophilus fungi. Truffles and macrofungi were considered to be diet items (Supporting information).

For the COI marker for metazoans, taxonomy was assigned by subjecting ASVs to a BLAST search against the NCBI nucleotide non-redundant database. A match to a Metazoan taxon with a minimum of 97% identity and 90% coverage was required for successful assignment; all ASVs failing to meet this threshold were considered non-target amplifications. Metazoan ASVs classified as annelids or insects were considered diet items. The metazoan marker also recovered a number of vertebrate taxa, including common shrew *Sorex araneus* in one individual, roe deer in four individuals, moose in four individuals, red deer in two individuals, salmon *Salmo salar* in eight individuals, and turkey *Gallus gallus* in a single individual. Although no vertebrate species were detected in negative DNA isolation controls, human *Homo sapiens* DNA was detected at low levels from 14 samples, suggesting potential for contamination in the field or lab. With the exception of the common shrew (and turkey), all of the vertebrate taxa detected are either routinely analysed in the same laboratory that processed these samples, or are species also harvested by the hunters that provided these samples. As such, all vertebrate taxa were considered to be potential contaminants and were excluded from further diet analysis. However it must be noted that these taxa accounted for < 10% of non-host sequences recovered in all but four of the individuals analyzed and are unlikely to represent significant components of the wild boar diet in this study.

Statistical analysis

All statistical analyses were conducted in the R ver. 4.2.2 statistical environment (www.r-project.org). For each sample, we calculated the proportion of the total sequences generated for the plant marker that were assigned to the target Streptophyta group, the proportion of the total fungal sequences generated that were assigned to fungal taxa considered to be diet items, and the proportion of the total non-host metazoan sequences generated that were assigned to annelid or arthropod taxa considered to be diet items. If none of these three categories exceeded 20% of the sequences generated, we considered it an indication of poor sample quality and removed the sample from further analyses (Supporting information, nine individuals). The four dispersers were removed from NMDS, PERMANOVA and general linear models (GLM) analyses both due to small sample number and in order to allow robust exploration of geographic region as an explanatory factor.

Overall patterns in wild boar winter diet composition were explored in relation to the focal explanatory variables of sex, body weight, region (south, north), Julian date, snow depth, and season (fall ≤ 25 cm snow, winter ≥ 25 cm snow) using NMDS and PERMANOVA analyses. Body weight was log-transformed in all analyses and an additional three individuals were excluded from these analyses due to missing snow depth or body weight data resulting in a final sampling size of 37. A proportional abundance diet matrix was assembled for NMDS and PERMANOVA analyses that consisted of the per-sample proportional abundance of each plant species relative to the total number of sequences generated by the plant marker, the per-sample proportional abundance of each fungal diet item relative to the total number of sequences generated by the fungal marker, and the per-sample proportional abundance for each metazoan genus relative to the total number of non-host sequences generated by the metazoan marker. As few of the individual diet items were consumed by

multiple individuals, we then aggregated this matrix into the following broad types of diet items: grass, herb, shrub, hypogeous fungi, epigeous fungi, insect, annelid, and gastropod. NMDS analysis was conducted on both of these matrices and the *envfit* function in 'vegan' was used to fit the explanatory variables to the NMDS axes. PERMANOVA analyses with forward model selection of all variables and their first order interactions was further used to find the best models explaining variation in winter diet composition. Models were evaluated using the Akaike Information Criterion corrected for small sample sizes (AICc) as implemented in the 'AICcPermanova' package in R and a threshold of −2 ΔAICc for a model to be considered a significant improvement on the null model (Corcoran 2023).

As hypogeous fungi (truffles) were identified as a major diet component whose abundance varied substantially between individuals, we used GLM with a negative binomial distribution to examine the effects of the focal variables (sex, body weight, region (south, north), Julian date, snow depth and season) on wild boar consumption of truffles. Models were evaluated using forward model selection using AICc as described above.

To investigate whether consumption of diet items with presumed human origin varied across time, region, and with individual traits, we used a GLM with a quasibinomial distribution with observations weighted according to the proportion of plant sequences recovered using the plant marker. Forward model selection of all variables and the first order interactions of any variable providing a significant improvement over the null model. Models were evaluated against one another using ANOVA to compare each model to the null model. All plants were classified as 'presumed human origin' (horticultural or agricultural species), 'presumed wild' (species native to the local ecosystem), or 'mixed' (both grown in agricultural settings, but also native to the local ecosystem) (Supporting information). Models were fitted for both the proportion of plant sequences of presumed human origin, as well as for the proportion of plant sequences of mixed or presumed human origin. As both analyses were concurrent, only the results for the proportion of plant sequences of presumed human origin are presented here.

Results

Sequencing performance

The plant marker generated 96 640 963 sequences, of which 39% were high quality and could be merged into non-chimeric full-length amplicons. Of these, 21 689 178 (57%) could be assigned to plant taxa. For the fungal marker, a total of 167 337 740 sequences were generated, of which 74% passed quality filtering and were merged into full length amplicons. Of the 119 570 639 non-chimeric sequences assembled, 97% (115 402 634) could be assigned to fungal taxa. A total of 12 821 968 sequences were generated from the COI metazoan marker, of which 50% were high quality and merged into full length amplicons. An additional 97 732 suspected chimeric sequences were removed, and the remaining 6 283 697 sequences (35%) were assigned to metazoan taxa. For all markers, the per-individual sequencing depth was sufficient to give asymptotic species accumulation curves (data not shown).

Description of diet composition

There was an extremely high degree of diet variability between individuals with most diet items (59%) being detected in only a single individual, and 86% of diet items being detected in three or fewer individuals (Fig. 2, Supporting information). Truffles were a frequent and abundant dietary item, with 36 of 50 individuals having consumed one or more species of these fungi. Frequently consumed plants included *Betula* spp., *Luzula* sp., *Helianthus* sp., *H. vulgare*, *Poa* sp., *Potentilla erecta*, *Solanum tuberosum*, *T. aestivum*, *Vaccinium* spp., all of which were consumed by six or more individuals. However, some of these (e.g. *Luzula* sp.) were consumed in relatively small amounts (mean 0.2% proportional abundance) compared to other diet items. A few plants (ex. *Oxalis acetosella*, *Taraxacum* sp., *Trifolium repens*) were consumed in relatively high amounts by some individuals (mean 21.3% proportional abundance). Very few metazoan diet items were recovered and no metazoan taxa occurred in > 4 individuals. Overall, 18, five and two individuals consumed insects, worms, and slugs or snails, respectively (Fig. 2, Supporting information). The majority of taxa detected were species native to Norway and represented plausible diet items. Among the 84 plant taxa detected, all represented known native species or known horticultural imports. Among the 201 fungal taxa detected, sixteen have not previously been reported in Norway according to the Norwegian Biodiversity Information Center, but have known European distributions. These taxa are nonetheless retained in the dataset due to the fact that fungal distributions are generally poorly circumscribed. All metazoan genera detected represent taxa with known Norwegian distributions.

Analysis of diet composition

NMDS ordinations exploring diet similarities between individuals captured primarily the consumption of increasing proportions of truffles consumed along the first axis (Supporting information), and found significant correlations between the ordination structure and both snow depth (R^2 = 0.261, p = 0.006, Fig. 3) and body weight (R^2 = 0.205, p = 0.023). PERMANOVA analyses of total diet composition using the five focal variables and all their first order interactions yielded no models with improved fit (ΔAICc > 2) as compared to the null model (Supporting information). PERMANOVA analyses of the matrix aggregated into functional groups (mushroom, truffle, herb, grass, shrub, insect, worm, slugs/snails) yielded near identical results (Supporting information). We also fitted binomial GLMs to explain the proportion of truffles recovered by the fungal marker, and although the proportion of truffles consumed declined with snow depth, none of the fitted models provided a significant improvement over the null model (Supporting information).

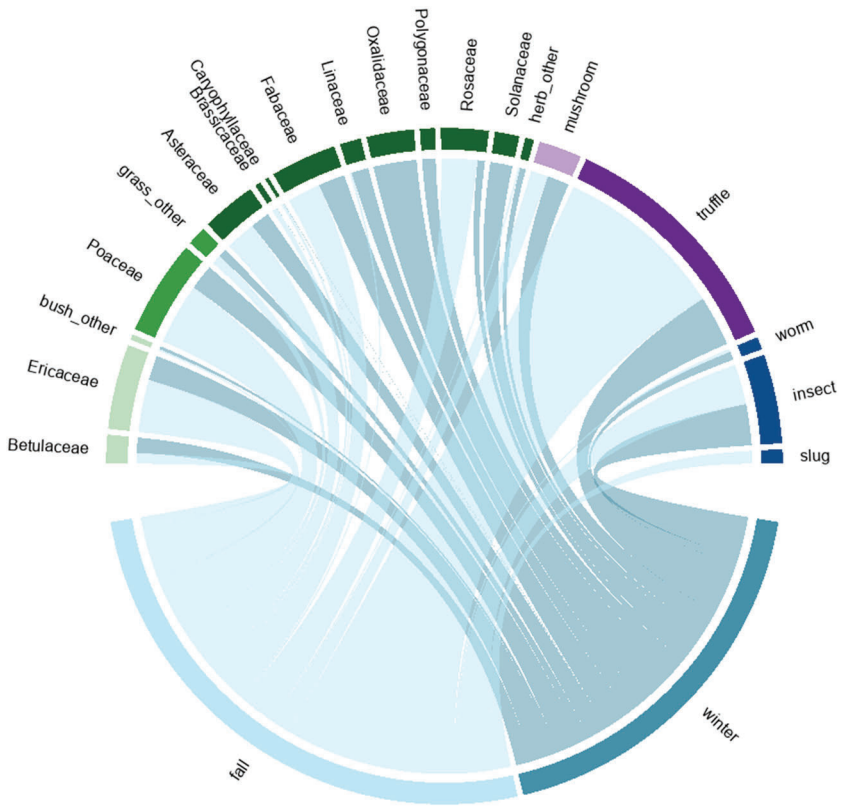

Figure 2. Diet composition of wild boars in Norway during the fall and winter periods as defined by snow depth. The circlize plot maps the seasons to the plant, fungal, and metazoan diet components detected using metabarcoding. Plant data is summarized at the family level and those families with < 10% maximum abundance and occurring in < 3 individuals are aggregated into the 'other' category for each of the growth forms shrub, grass, and herb.

Diet with human origin

Diet items that were of presumed human origin included wheat, barley, and potato, which were detected in 8, 19 and 11 individuals, respectively. Less frequently consumed diet items of human origin included soy *Glycine max*, sunflower *Helianthus annuus*, red beet *Beta vulgaris* and rye (Supporting information). Model selection identified that two single factor models with sex and body weight provided a significantly better fit to the data than the null model (Supporting information), and the model including sex, weight, and their interaction was identified as providing a significant improvement in fit (Table 2). In the best fitted model, the proportion of plants consumed with human origin increased significantly with animal body weight ($p = 0.048$), and there was weak evidence for an interaction between sex and weight ($p = 0.060$). Heavy, male individuals consumed more plants of presumed human origin compared to lighter, females (Fig. 4).

Discussion

With increasing concern over wild boar impact on biodiversity and crops in some regions, and threat of ASF (Fulgione and Buglione 2022), limiting wild boar distribution is a key issue to management. Diet quality is a main determinant of wild boar population dynamics (Servanty et al. 2009, Gamelon et al. 2017), and winter conditions are assumed to be limiting for large mammal populations at northern distribution ranges (Klein 1965). Understanding the extent to which wild boar rely on high quality human-provided food sources that may be available during resource limited times-of-year could provide a key to limiting their distribution. Using a metabarcoding approach, we provide the first study of wild boar fall–winter diet in Norway. Our dataset was limited to 50 samples, but clear patterns nevertheless emerged. Fungi were clearly an important diet item during both winter and fall. We found no oak *Quercus robur* in wild boar diet. Small amounts of maize were consumed infrequently. Furthermore, all metazoan taxa (including vertebrate taxa) were detected infrequently and in relatively low amounts suggesting they are not systematically consumed as a part of the wild boar fall–winter diet. There was large individual heterogeneity in diet species composition, and the (heavier) males consumed higher proportions of human-provided food sources than the (lighter) females. Further studies with larger sample sizes would be required for detecting more minor variation in diet, and we highlight some relevant methodological limitations and challenges.

Truffles important, but affected by snow depth

A review of wild boar diet in western Europe identified mast, roots, green plant matter and agricultural crops as the four

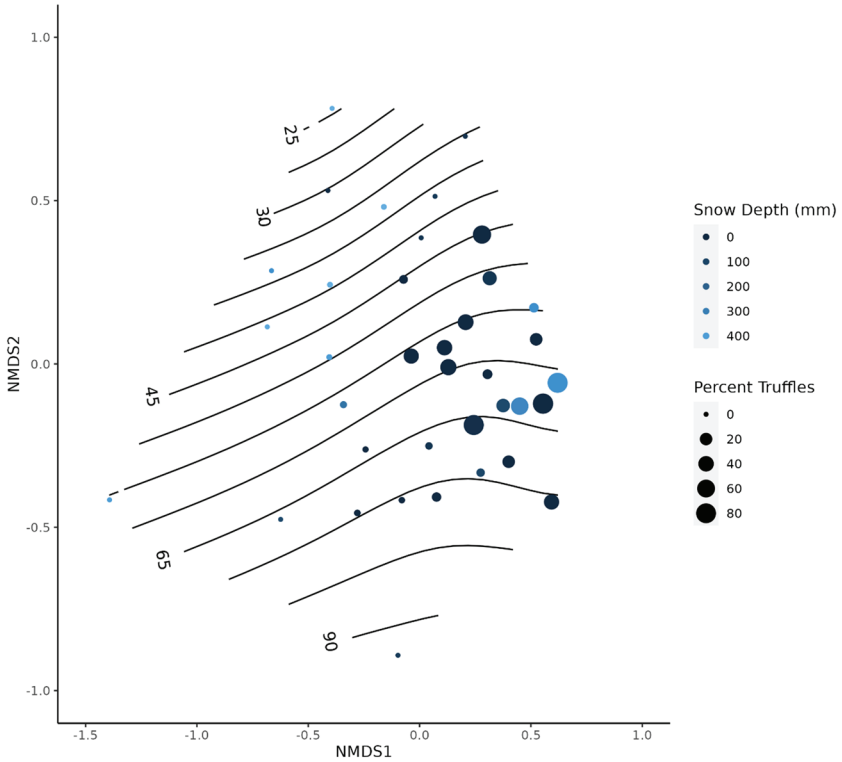

Figure 3. NMDS ordination showing similarity in diet composition between individuals. Points are sized according the proportion of truffles consumed, and coloured according to the snow depth at the time the animal was harvested. Contour lines show variation in body weight of the individuals analysed.

major vegetable food categories (Schley and Roper 2003). Our results suggest wild boars feed frequently on mushrooms, particularly those with hypogeous growth forms (truffles) for parts of the fall–winter period. Truffles were reported to be an important part of the wild boar diet in coniferous forests in Poland (Lawrynowicz et al. 2006), and are also frequently eaten by wild boars in Mediterranean oak forests (Piattoni et al. 2012, Piattoni et al. 2016). During surveys of rooting in Norway (Haaverstad et al. 2014), authors noted truffles in the faeces of wild boar, and our study confirms this to be the case. Mushrooms are considered to be readily digestible and with a high sugar content, and might be an important diet component in these areas lacking acorns, rich crops and intentional artificial feeding. Similarly, fungi were found in the fall diet of moose and roe deer in Sweden based on rumen analysis, but in fairly small (up to 10%) proportions (Cederlund et al. 1980). Free-ranging domestic sheep *Ovis aries* in Norway were very keen to find mushrooms and it largely affects their pattern of ranging in fall (Warren and Mysterud 1991). Consumption of truffles by wild boar generally declined with snow depth, but their detection in a few late season individuals during periods with significant snow cover may be explained by local micro-conditions, as snow depth is lower under tree canopies where truffles are expected to fruit. Truffles hence appear to be an important, high quality natural diet item, and may be important for wild boar survival in these northern forests.

Table 2. Summary of the three best fitted GLMs for the proportion of plants with presumed human origin consumed by wild boars in fall and winter (n = 39) in Norway. Baseline level for sex was male.

Model	Variable	Estimate	SE	t	p
no. 1	Sex	−1.470	0.507	−2.891	0.007
no. 2	Weight	1.017	0.527	1.930	0.063
no. 3	Sex	6.472	4.067	1.592	0.122
	Weight	1.632	0.789	2.067	0.048
	Sex:weight	−1.849	0.945	−1.957	0.060

Absence of oak and low frequency of maize in diet

Oak acorns are a preferred dietary item for wild boar during autumn and winter in the Czech Republic (Mikulka et al. 2018) and in Italy (Massei et al. 1996). Oak forests with the potential to provide acorns as a food resource for wild boars are restricted to a narrow distribution along the southern coastline of Norway (Abrahamsen et al. 1977), and we found no oak in the wild boar diet in Norway. Grasses are a frequent component of wild boar diet across Europe (Rutten et al. 2019, Spitzer et al. 2020, Petrelli et al. 2022), and we also found several grass species frequently consumed by Norwegian individuals. While fruits and seeds were more common in their diet in deciduous forests, wild boar ate more woody browse in coniferous forests during winter (Spitzer et al. 2020). We did not observe frequent consumption or large proportions of woody species (Betulaceae, Ericaceae) during

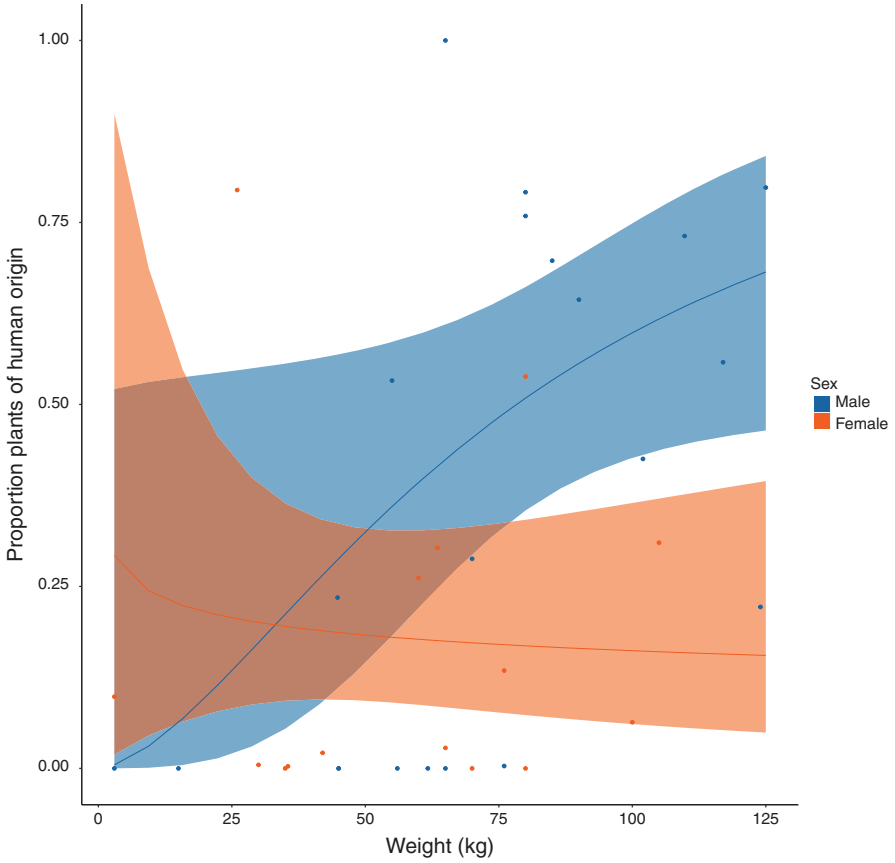

Figure 4. Effects plot showing the relationship between proportion of plants with presumed human origin consumed and weight for male and female wild boars. The (heavier) males ate more human origin food than the (lighter) females, but there was only weak evidence for an interaction term between sex and weight.

winter, but it must be noted that the genetic primers used here perform poorly on conifers. Browsing of these plants may remain undetected (De Barba et al. 2014, Tercel et al. 2021) and any consumption of pine seeds, an energy rich food item from a masting species that wild boar are known to consume in Mediterranean forests (Massei et al. 1996, Schley and Roper 2003), may be missed. Rooting activity of wild boar in Norway was common in pine forest during winter (Haaverstad et al. 2014), and further studies are needed to determine whether pine browse or seeds form a part of the winter diet of wild boar in Norway.

Maize is a preferred food item for wild boar in many areas, including Flanders (Rutten et al. 2019), Poland (Piekarczyk et al. 2021), and the Czech Republic (Mikulka et al. 2018). Maize is not a common agricultural crop in Norway, but maize is used by hunters to attract wild boar. In Norway, feeding of wild boar is banned, while 'baiting' is allowed (Norwegian Environment Agency and Norwegian Food Safety Authority 2019). Baiting is defined as a limited supply of food to about 10 kg km^{-2} $month^{-1}$. Baiting is a commonly used technique for hunters to attract and shoot wild boar. However, the extent to which these food ration targets are followed remain uncertain, and it may be difficult to determine when baiting becomes feeding. It was therefore interesting to note the low frequency of maize in the diet of wild boar, providing evidence that maize does not appear to be a large part of their diet in these areas despite its use in baiting. The most common human origin dietary items in Norway were wheat, barley, and potato, while wild boar less frequently consumed soy, sunflower, red beet, and rye. This is fairly similar to Sweden, where wild boar were reported to select areas for crop fields with oat, spring wheat and mixed crops (Muthoka et al. 2022). The origin of at least wheat may also stem from baiting with bread.

Individual heterogeneity in diet

Few studies on wild boar address the extent of dietary variation between individuals. A clear result of our study is the large individual heterogeneity in species composition in the fall–winter diet of wild boar in Norway. We found no evidence that diet composition overall was structured by sex, age or body weight in wild boar. However, heavier males tended to consume more plants that are presumed to be of human origin (Fig. 5). These plant species tend to be agricultural plants and hence available in open habitat. Use of open agricultural habitat by deer come with higher risk of being shot by hunters (Norum et al. 2015), and this is likely perceived as more risky habitat also for wild boar. Hence, male wild boar eating more human origin food than females

may indicate differences in risk sensitivity, similar to other ungulates (Bleich et al. 1997).

Overall, the large individual variation in diet may reflect opportunistic feeding tactics in boreal forests during fall and winter. However, the variance may also reflect the short time window of observation in faecal samples. Based on studies of domestic pigs, wild boar gut retention times are expected to be relatively short, at approximately two days (Davis et al. 2001, Wilfart et al. 2007, Henze et al. 2021). This, coupled with a lack of repeated sampling of individuals likely contributes to a high level of variability, with the vast majority of diet items being consumed only by a single individual and few trends in overall diet composition.

Methodological considerations

As outlined in the introduction, both faecal based diet analyses and the metabarcoding methodology are subject to biases and limitations, and omnivore diets have been recognized as uniquely challenging to characterize using metabarcoding (De Barba et al. 2014, Tercel et al. 2021). Unsurprisingly, the data presented here includes examples of a number of these biases and limitations. There were signs of 'accidental bycatch' taxa being consumed during foraging, as many microscopic soil fungi, rotifers and tardigrades were detected, but are unlikely to represent significant energetic components of the wild boar diet. In addition, while wild boar can exhibit scavenging behaviour (Carpio et al. 2023), the samples used in this study were provided by hunters, and we cannot unequivocally determine if the presence of commonly harvested taxa like roe deer, red deer, and moose represent cadaver consumption or if DNA from these species was introduced from hunting implements previously used in the harvest of these species. Similarly, the detection of salmon in eight individuals at proportional abundances < 2% could represent foraging of human garbage by wild boar, or may represent low level laboratory contamination, as samples were handled in a lab that routinely also isolates DNA from salmon.

With regards to the metabarcoding methodology, primer bias and limitations can also significantly impact results (De Barba et al. 2014, Tercel et al. 2021). The primers used in this study do not amplify members of the plant family Pinaceae (Cheng et al. 2016), potentially missing important diet items, as discussed above. Primer selection for characterizing omnivorous diets is challenging, as there is typically an inverse relationship between the taxonomic breadth a primer targets, and the taxonomic resolution it is able to provide for those groups it is targeting. As such primer selection can become a trade-off between achieving taxonomic breadth, which allows direct comparisons of abundance between diet items, and maintaining taxonomic resolution which allows species-level identifications of diet items (Tercel et al. 2021). The three markers used in this study provide good resolution for distinguishing between plant, metazoan, and fungal taxa, and successfully recover the broad dietary diversity found in the Scandinavian wild boar winter diet. However, direct comparisons of abundance are only possible within each of these taxa, and not between them. Nevertheless, we observed that individuals in which a low total proportion of plant sequences were recovered frequently had a high proportion of edible fungi, and vice versa, confirming that an absence of plant read or fungal sequences was not universally indicative of technical problems in sequencing a faecal sample, and can provide some coarse measure of an individual's relative consumption of these diet items. Our results illustrate that while DNA-based dietary analysis provides valuable high-resolution data, the results must be interpreted with caution, and in light of all possible contamination scenarios and methodological biases.

Conclusion

We currently lack insight on how well wild boar are adapted to the northern ecosystems of Scandinavia, comprised of forests without acorns and with few rich agricultural crops. We documented large individual heterogeneity in species composition in the winter diet of wild boar in Norway. Truffles were an important part of diet, but intake was reduced with increasing snow depth. Further studies with larger sample sizes and year-round sampling are required to determine how different legal feeding regimes allowed by management in Norway compared to Sweden impact wild boar diet, but the low frequency of maize in diet of wild boar at least suggests compliance to the ban on feeding. Further, it implies that hunting using baiting (with maize), being an effective way to shot wild boar, appear not to markedly affect wild boar diet. Such knowledge will be valuable to target efforts to effectively limit wild boar given the management aim to keep 'the fewest possible wild boar in the minimum possible range' (Norwegian Environment Agency and Norwegian Food Safety Authority 2019).

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Author contributions

Atle Mysterud: Conceptualization (equal); Visualization (supporting); Writing – original draft (lead). **Marie Davey:** Formal analysis (lead); Methodology (lead); Visualization (lead); Writing – original draft (supporting). **Frode Fossøy:** Formal analysis (supporting); Writing – review and editing (equal). **Carl Andreas Grøntvedt:** Data curation (supporting); Writing – review and editing (equal). **Christer M. Rolandsen:** Conceptualization (equal); Data curation (lead); Funding acquisition (lead); Project administration (lead); Writing – review and editing (equal).

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Data availability statement

Data are available from the Zenodo Repository: https://doi.org/10.5281/zenodo.10571525 (Mysterud et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Abrahamsen, J., Jacobsen, N. K., Kalliola, R., Dahl, E., Wilborg, L. and Påhlsson, L. 1977. Naturgeografisk region-inndeling av Norden. – Nordiske Utredninger Ser. B 34: 1–135.

Artsdatabanken. 2015. Artsnavnebasen. Norsk Taksonomisk database. – http:. www.artsportalen.artsdatabanken.no/.

Ballari, S. A. and Barrios-García, M. N. 2014. A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. – Mamm. Rev. 44: 124–134.

Banchi, E., Ametrano, C. G., Greco, S., Stanković, D., Muggia, L. and Pallavicini, A. 2020. PLANiTS: a curated sequence reference dataset for plant ITS DNA metabarcoding. – Database 2020: baz155.

Bischof, R., Milleret, C., Dupont, P., Chipperfield, J., Tourani, M., Ordiz, A., de Valpine, P., Turek, D., Royle, J. A., Gimenez, O., Flagstad, Ø., Åkesson, M., Svensson, L., Brøseth, H. and Kindberg, J. 2020. Estimating and forecasting spatial population dynamics of apex predators using transnational genetic monitoring. – Proc. Natl Acad. Sci. USA 117: 30531.

Bleich, V. C., Bowyer, R. T. and Wehausen, J. D. 1997. Sexual segregation in mountain sheep: resources or predation? – Wildl. Monogr. 134: 1–50.

Bull, J., Nilsen, E. B., Mysterud, A. and Milner-Gulland, E. J. 2009. Survival on the border: a population model to evaluate management options for Norway's wolves. – Wildl. Biol. 15: 412–424.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. and Holmes, S. P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. – Nat. Methods 13: 581.

Carpio, A. J., Queirós, J., Laguna, E., Jiménez-Ruiz, S., Vicente, J., Alves, P. C. and Acevedo, P. 2023. Understanding the impact of wild boar on the European wild rabbit and red-legged partridge populations using a diet metabarcoding approach. – Eur. J. Wildl. Res. 69: 18.

Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A. and Hebert, P. D. N. 2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. – PLoS One 6: e22232.

Cederlund, G., Ljungqvist, H., Markgren, G. and Stålfelt, F. 1980. Foods of moose and roe deer at Grimsö in central Sweden – results of rumen content analysis. – Swed. Wildl. Res. 11: 167–247.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. and Leon, C. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. – PLoS One 5: e8613.

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y. and Zhou, S. 2016. Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. – Mol. Ecol. Resour. 16: 138–149.

Corcoran, D. 2023. AICcPermanova: model selection of PERMANOVA models using AICc. – R package ver. 0.0.2, https://CRAN.R-project.org/package=AICcPermanova.

Cretois, B., Linnell, J. D. C., Grainger, M., Nilsen, E. B. and Rød, J. K. 2020. Hunters as citizen scientists: contributions to biodiversity monitoring in Europe. – Global Ecol. Conserv. 23: e01077.

Davis, S. S., Illum, L. and Hinchcliffe, M. 2001. Gastrointestinal transit of dosage forms in the pig. – J. Pharm. Pharmacol. 53: 33–39.

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E. and Taberlet, P. 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. – Mol. Ecol. Resour. 14: 306–323.

de Sousa, L. L., Silva, S. M. and Xavier, R. 2019. DNA metabarcoding in diet studies: unveiling ecological aspects in aquatic and terrestrial ecosystems. – Environ. DNA 1: 199–214.

Dixon, L. K., Stahl, K., Jori, F., Vial, L. and Pfeiffer, D. U. 2020. African swine fever epidemiology and control. – Annu. Rev. Anim. Biosci. 8: 221–246.

Fulgione, D. and Buglione, M. 2022. The boar war: five hot factors unleashing boar expansion and related emergency. – Land 11: 887.

Fulgione, D., Rippa, D., Buglione, M., Trapanese, M., Petrelli, S. and Maselli, V. 2016. Unexpected but welcome. Artificially selected traits may increase fitness in wild boar. – Evol. Appl. 9: 769–776.

Gamelon, M., Focardi, S., Baubet, E., Brandt, S., Franzetti, B., Ronchi, F., Venner, S., Sæther, B. E. and Gaillard, J. M. 2017. Reproductive allocation in pulsed-resource environments: a comparative study in two populations of wild boar. – Oecologia 183: 1065–1076.

Haaverstad, O., Hjeljord, O. and Wam, H. K. 2014. Wild boar rooting in a northern coniferous forest - minor silviculture impact. – Scand. J. Forest Res. 29: 90–95.

Henze, L. J., Koehl, N. J., Bennett-Lenane, H., Holm, R., Grimm, M., Schneider, F., Weitschies, W., Koziolek, M. and Griffin, B. T. 2021. Characterization of gastrointestinal transit and luminal conditions in pigs using a telemetric motility capsule. – Eur. J. Pharm. Sci. 156: 105627.

Herrero, J., García-Serrano, A., Couto, S., Ortuño, V. M. and García-González, R. 2006. Diet of wild boar *Sus scrofa* L. and crop damage in an intensive agroecosystem. – Eur. J. Wildl. Res. 52: 245–250.

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E. and Lindahl, B. D. 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. – FEMS Microbiol. Ecol. 82: 666–677.

Jonsson, L. 1986. From wild boar to domestic pig – a reassessment of Neolithic swine of northwestern Europe. – In: Königsson, L. K. (ed.), Nordic late quaternary biology and ecology – striae, vol. 24. Archaeology Data Service, pp. 25–129.

Klein, D. R. 1965. Ecology of deer range in Alaska. – Ecol. Monogr. 35: 259–284.

Lawrynowicz, M., Falinski, J. B. and Bober, J. 2006. Interactions among hypogeous fungi and wild boars in the subcontinental pine forest. – Biodivers. Res. Conserv. 1–2: 102–106.

Lemel, J., Truvé, J. and Söderberg, B. 2003. Variation in ranging and activity behaviour of European wild boar *Sus scrofa* in Sweden. – Wildl. Biol. 9: 29–36.
Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T. and Machida, R. J. 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. – Front. Zool. 10: 34.
Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. – EMBnet J. 17: 10–13.
Massei, G., Genov, P. V. and Staines, B. W. 1996. Diet, food availability and reproduction of wild boar in a Meditteranean coastal area. – Acta Theriol. 41: 307–320.
Massei, G., Roy, S. and Bunting, R. 2019. Too many hogs? A review of methods to mitigate impact by wild boar and feral hogs. – Hum. Wildl. Interact. 5: 79–99.
Mikulka, O., Zeman, J., Drimaj, J., Plhal, R., Adamec, Z., Kamler, J. and Heroldová, M. 2018. The importance of natural food in wild boar (*Sus scrofa*) diet during autumn and winter. – Folia Zool. 67: 165–172.
Molia, A., Larsson, E., Jeppson, M., Læssøe, T. and Larsson, K. H. 2020. *Elaphomyces* section *Elaphomyces* (Eurotiales, Ascomycota) – taxonomy and phylogeny of north European taxa, with the introduction of three new species. – Fungal Syst. Evol. 5: 283–300.
Muthoka, C. M., Andren, H., Nyaga, J., Augustsson, E. and Kjellander, P. 2022. Effect of supplemental feeding on habitat and crop selection by wild boar in Sweden. – Ethol. Ecol. Evol. 35: 1–19.
Mysterud, A., Davey, M., Fossøy, F., Grøntvedt, C. A. and Rolandsen, C. M. 2024. Data from: Using DNA metabarcoding to separate natural and human provided food in wild boar diet at the northern distribution range of Europe. – Zenodo Repository, https://doi.org/10.5281/zenodo.10571525.
Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S. and Kennedy, P. G. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. – Fungal Ecol. 20: 241–248.
Nilsson, R. H. et al. 2018. Taxonomic annotation of public fungal ITS sequences from the built environment - a report from an April 10–11, 2017 workshop (Aberdeen, UK). – MycoKeys 28: 65–82.
Norum, J. K., Lone, K., Linnell, J. D. C., Odden, J., Loe, L. E. and Mysterud, A. 2015. Landscape of risk to roe deer imposed by lynx and different human hunting tactics. – Eur. J. Wildl. Res. 61: 831–840.
Norwegian Biodiversity Information Center. 2023. Truffles. – Trøfler67-10 (artsdatabanken.no).
Norwegian Environment Agency and Norwegian Food Safety Authority. 2019. Action plan to reduce wild boar distribution in Norway 2020–2024. – Norwegian Environment Agency.
Odden, J., Thorsen, N. H., Bahlk, S., Rolandsen, C. M., Rivrud, I. M. and Rosvold, J. 2023. Population monitoring of wild boar – status 2022. – NINA rapport 2293, pp. 1–19.
Palencia, P., Blome, S., Brook, R. K., Ferroglio, E., Jo, Y. S., Linden, A., Montoro, V., Penrith, M. L., Plhal, R., Vicente, J., Viltrop, A. and Gortázar, C. 2023. Tools and opportunities for African swine fever control in wild boar and feral pigs: a review. – Eur. J. Wildl. Res. 69: 69.
Petrelli, S., Buglione, M., Maselli, V., Troiano, C., Larson, G., Frantz, L., Manin, A., Ricca, E., Baccigalupi, L., Wright, D., Pietri, C. and Fulgione, D. 2022. Population genomic, olfactory, dietary, and gut microbiota analyses demonstrate the unique evolutionary trajectory of feral pigs. – Mol. Ecol. 31: 220–237.
Piattoni, F., Oir, F., Morara, M., Iotti, M. and Zambonelli, A. 2012. The role of wild boars in spore dispersal of hypogeous fungi. – Acta Mycol. 47: 145–153.
Piattoni, F., Ori, F., Amicucci, A., Salerni, E. and Zambonelli, A. 2016. Interrelationships between wild boars (*Sus scrofa*) and truffles. – In: Zambonelli, A., Iotti, M., Murat, C. (eds), True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Springer International Publishing, pp. 375–389.
Piekarczyk, P., Tajchman, K., Belova, O. and Wójcik, M. 2021. Crop damage by wild boar (*Sus scrofa* L.) depending on the crop composition in central-eastern Poland. – Baltic For. 27: 552.
Putman, R. J. 1984. Facts from faeces. – Mamm. Rev. 14: 79–97.
Robeson, M. S., M.S., Khanipov, K., Golovko, G., Wisely, S. M., White, M. D., Bodenchuck, M., Smyser, T. J., Fofanov, Y., Fierer, N.. and Piaggio, A. J. 2018. Assessing the utility of metabarcoding for diet analyses of the omnivorous wild pig (*Sus scrofa*). – Ecol. Evol. 8: 185–196.
Rognes, T., Flouri, T., Nichols, B., Quince, C. and Mahé, F. 2016. VSEARCH: a versatile open source tool for metagenomics. – PeerJ 4: e2584.
Rosvold, J. and Andersen, R. 2008. Wild boar in Norway – is climate a limiting factor? – NTNU Vitenskapsmuseet Rapp Zool. Ser. 2008: 1–23.
Rosvold, J., Halley, D. J., Hufthammer, A. K., Minagawa, M. and Andersen, R. 2010. The rise and fall of wild boar in a northern environment: evidence from stable isotopes and subfossil finds. – Holocene 20: 1113–1121.
Rutten, A., Casaer, J., Strubbe, D. and Leirs, H. 2019. Agricultural and landscape factors related to increasing wild boar agricultural damage in a highly anthropogenic landscape. – Wildl. Biol. 2020: wlb.00634.
Schley, L. and Roper, T. J. 2003. Diet of wild boar *Sus scrofa* in western Europe, with reference to consumption of agricultural crops. – Mamm. Rev. 33: 43–56.
Servanty, S., Gaillard, J.-M., Allainé, D., Brandt, S. and Baubet, E. 2007. Litter size and fetal sex ratio adjustment in a highly polytocous species: the wild boar. – Behav. Ecol. 18: 427–432.
Servanty, S., Gaillard, J.-M., Toïgo, C., Serge, B. and Baubet, E. 2009. Pulsed resources and climate-induced variation in the reproductive traits of wild boar under high hunting pressure. – J. Anim. Ecol. 78: 1278–1290.
Spitzer, R., Felton, A., Landman, M., Singh, N. J., Widemo, F. and Cromsigt, J. P. G. M. 2020. Fifty years of European ungulate dietary studies: a synthesis. – Oikos 129: 1668–1680.
Tercel, M. P. T. G., Symondson, W. O. C. and Cuff, J. P. 2021. The problem of omnivory: a synthesis on omnivory and DNA metabarcoding. – Mol. Ecol. 30: 2199–2206.
Vercauteren, K. C., Beasley, J. C., Ditchkoff, S. S., Mayer, J. J., Roloff, G. J. and Strickland, B. K. 2020. Invasive wild pigs in North America: ecology, impacts, and management. – Taylor & Francis Group.
Vicente, J. et al. 2019. Science-based wildlife disease response. – Science 364: 943.
VKM, Skjerve, E., Thurfjell, H., Fløe, D., Grahek-Ogden, D., Malmstrøm, M., Nesbakken, T., das Neves, C., Nielsen, A., Pedersen, H. C., Robertson, L., Rueness, E. K., de Boer, H., Gudding, R., Hoel, K., Kirkendall, L., Vandvik, V. and Wasteson, Y. 2018. Wild boar population growth and expansion in Norway - Implications for biodiversity, food safety

and animal health in Norway. Opinion of Norwegian scientific committee for food and environment. – VKM report 2018, vol. 14. Norwegian Scientific Committee for Food and Environment, Oslo.
Warren, J. T. and Mysterud, I. 1991. Fungi in the diet of domestic sheep. – Rangelands 13: 168–171.
White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogentics. – In: Innis, M. A., Gelfland, D. H., Sninsky, J. J. and White, T. J. (eds), PCR protocols: a guide to methods and applications. Academic Press, pp. 315–322.
Wilfart, A., Montagne, L., Simmins, H., Noblet, J. and Milgen, J. v. 2007. Digesta transit in different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by wheat bran. – Br. J. Nutr. 98: 54–62.
Wilson, C. J. 2004. Rooting damage to farmland in Dorset, southern England, caused by feral wild boar *Sus scrofa*. – Mamm. Rev. 34: 331–335.